the serving of unpasteurized egg products unless fully cooked. In fact, since as early as 1969, the USDA has overseen the processing of liquid egg removed from the shell to reduce the level of *Salmonella* contamination to acceptable levels. However, no commercially acceptable methods have been developed to combat *Salmonella* in shell eggs. Since shell eggs must be used in situations where a liquid egg product cannot, it is therefore desirable to develop a commercially acceptable process for the reduction of *Salmonella* within shell eggs to provide a safe and functionally acceptable shell egg to the consumer.

Thermal treatments of shell egg to prevent embryonic growth in fertile eggs, to reduce incidence of spoilage during long term storage, and maintain internal quality received considerable research attention from about 1943 to about 1953. This research resulted from the nature of the egg industry at that time in that most of the eggs were produced by small flocks and the majority of the eggs used by the food industry were collected as seasonal surpluses in the spring. As a result of the production practices the eggs were more likely to lose interior quality or become unfit for human consumption because of bacterial growth or embryonic development. Research into "thermostabilization" was directed at solving these problems, which were largely perceived as embryonic growth and the contamination of the egg from contaminants external to the shell. (See Egg Science, Stadelman and Coterill, (eds.), Chapter 4, 3d Ed., 1986).

U.S. Pat. No. 2,423,233 to Funk describes the thermostabilization of shell eggs. The '233 patent described a process of heating the shell egg to arrest embryonic development in the egg. As described in the '233 patent, when heating with water the preferred times and temperatures for the heat treatment were 138 degrees Fahrenheit for from five to ten minutes. However, the work of Dr. Funk was not concerned with the elimination of pathogenic organisms. In fact, the times and temperatures suggested by Dr. Funk for heating with water would not be sufficient to cause high enough levels of *Salmonella enteritidis* destruction to insure that a safe shell egg would result. Furthermore, because eggs available through modern production and

distribution are fresher and have a lower pH they require a different thermal process than was used by Funk.

Accordingly, it is one object of the present invention to provide a safe shell egg product which is essentially free of *Salmonella* and more preferably free of *Salmonella enteritidis*.

B2

It is another object of the present invention to provide a commercially acceptable process for reducing the levels of *Salmonella enteritidis* in shell eggs to acceptable levels.

It is still a further object of the present invention to provide a method of producing a *Salmonella* negative shell egg without requiring additional thermal treatments which could reduce the functionality of the shell egg. --

Please replace the paragraphs beginning at page 3, line 15, with the following rewritten paragraphs:

B3

-- The present invention provides methods for producing a pasteurized shell egg while retaining the normal appearance of the shell egg contents. The present invention, therefore, relates to a commercially viable method of producing a pasteurized shell egg. One particular embodiment of the present invention involves heating the shell egg in an aqueous solution of a predetermined temperature for a predetermined time. The heating at a predetermined time for a predetermined temperature provide to the albumen of the shell egg a total thermal treatment which can be described by an equivalent time and an equivalent temperature which define a point above the "Whites" line of Figure 1 but is insufficient to cause coagulation of either the albumen or the yolk of the shell egg.

In another aspect of the present invention the equivalent time and equivalent temperature define a point above the "Yolk" line of Figure 1, but again insufficient to cause coagulation of either the albumen or the yolk of the shell egg.--

Please replace the paragraphs beginning at page 4, line 15, with the following rewritten paragraphs:

--Yet another aspect of the present invention provides a method of producing a pasteurized shell egg by heating the shell egg in an aqueous solution of a predetermined temperature and maintaining the shell egg in the aqueous solution for a predetermined time, wherein the predetermined time and the predetermined temperature define a point above the "Apparent F_0" line of Figure 1, and wherein the predetermined time and the predetermined temperature are insufficient to cause coagulation of the albumen or the yolk of the shell egg. A further aspect of the present invention provides a thermal treatment wherein the predetermined time and the predetermined temperature define a point below the "Expected Salmonella" line of Figure 1.

B4

The present invention is also directed to a pasteurized shell egg, wherein the albumen of said shell egg has received a thermal treatment sufficient to cause a 9D reduction in *Salmonella enteritidis* but insufficient to cause significant coagulation. In another aspect of the thermally treated shell egg, the yolk of the shell egg receives a thermal treatment sufficient to cause a 9D reduction in *Salmonella enteritidis* but insufficient to cause coagulation. -

Please replace the paragraph beginning at page 5, line 8, with the following rewritten paragraph:

B5

--**Figure 1** is a graph of the apparent F_0 line superimposed on the thermal death time curves for *Salmonella*. --

Please replace the paragraphs beginning at page 5, line 28, with the following rewritten paragraphs:

B

-- One aspect of the present invention involves the heating of shell eggs in an aqueous solution of a specified temperature for a time sufficient to cause at least a reduction in *Salmonella enteritidis* (SE) of greater than 5 log cycles (5D). More preferably, the shell egg is placed in aqueous solution wherein the time in the solution and the temperature of the solution impart a treatment to the shell egg sufficient to cause a greater than 7D reduction in SE, and most preferably a reduction in SE of greater than 9D. It is preferred that the treatment of the shell egg be sufficient to cause the reduction in SE in the albumen of the shell egg and most preferable that the treatment be sufficient to cause the SE reduction in both the albumen and the yolk of the shell egg. These reductions in SE should be accomplished while retaining the functionality of the shell egg (e.g., maintaining the egg yolk and egg white in essentially liquid form).

B6

For comparative purposes, it is noted that PCT Application No. WO 93/03622 to Cox describes a method of "hyperpasteurization" of shell eggs. As is described in Figure 10 of Cox, relatively severe thermal treatments are expected to be required before *Salmonella* is destroyed. The data points shown in Figure 10 of Cox may be used to construct a line which reflects what would be an expected *Salmonella* destruction line for shell eggs. This "Expected Salmonella" line is labelled as such and is shown in Figure 1 herein ("Expected Salmonella") and has the equation $\log(t)=8.456-0.1183T$, where t is time in minutes and T is temperature in °C. However, these more severe thermal treatments could cause loss in functionality to the shell egg (e.g., partial or complete coagulation of the egg yolk or egg white). --

Please replace the paragraph beginning at page 7, line 1, with the following rewritten paragraph:

-- In the present invention, the thermal treatment employed preferably defines a point below the "Expected Salmonella" line of Figure 1. Furthermore, the treatment of the shell egg should be insufficient to cause

B7

coagulation of either the albumen or the yolk of the shell egg. The methods of the present invention result in a SE negative shell egg having essentially the natural proportion of indigenous gases.--

Please replace the paragraphs beginning at page 8, line 19, with the following rewritten paragraphs:

-- While lower temperatures may be used, in practice, aqueous solution temperatures of greater than about 134°F (or about 56°C) and less than about 140°F (or about 60°C) are preferred and, as discussed above, it is preferred that the temperature of the solution remain approximately constant for the time the shell eggs are heated. Times of from about 20 minutes to about 45 minutes or greater may be selected to achieve the desired reduction in *Salmonella* with shorter times being required for higher temperatures. The specific times and temperatures required may vary with size, age and pH of the shell egg and whether the shell egg has been oiled before or after thermal treatment.

If an equivalent point analysis of the thermal treatment received by a particular portion of the shell egg is utilized to determine the reduction of SE in the shell egg, then the resulting equivalent time and equivalent temperature should define a point above the desired *Salmonella* thermal death time curves such as those shown in Figure 2 and Table 6 of the USDA Egg Pasteurization Manual, ARS 74-38, Agricultural Research Service, United States Department of Agriculture, Albany, CA (1969) which are labelled as such and reproduced in Figure 1 herein and labelled as "Whites," "Yolk" and "Whole Egg".

If an F_0 analysis is employed in carrying out the present invention, then to assure a sufficient reduction in *Salmonella* such that no shell eggs test positive for *Salmonella* utilizing approved tests for *Salmonella*, such as those approved by the USDA for use in liquid egg processing and discussed in the Egg Pasteurization Manual, then actual time and temperature combinations which define points at or above both the "Apparent F_0" line and the

Salmonella thermal death time curve of Figure 1 should be utilized. As will be understood by one of skill in the art, variations in shell egg physical characteristics, such as size, age, pH, etc., may cause the shell egg "Apparent F_0" line of Figure 1 to shift. --

B8

Please replace the paragraph beginning at page 12, line 18, with the following rewritten paragraph:

--Table 1 presents the results of the thermal treatments on the survival of *S. enteritidis* inoculated into shell eggs. As temperature increased, the time required to obtain *Salmonella* negative eggs decreased. At 56°C, exposure time required to obtain no positive eggs was greater than 41 minutes. At 56.75 and 57.5°C, exposure times greater than 28 and 23 minutes, respectively, were required to obtain eggs negative for *Salmonella*. Standard USDA tests for *Salmonella* were utilized. --

B9

Please replace the paragraphs beginning at page 13, line 25, with the following rewritten paragraphs:

-- Times at temperatures where none of the twelve inoculated eggs were positive, were used in a regression equation to determine the thermal death time curve (TDTC) presented in Figure 1 as the "Apparent F_0" line. The equation for the line is:

$$\log (t) = -0.1216 \times T + 8.4274$$

B10

where t is the time in minutes and T is temperature in degrees Centigrade. The R^2=0.86.

The above equation may be considered a workable approximation or an "Apparent F_0" line for *S. enteritidis* in shell eggs. The temperature range

and times used to obtain the data were selected with the intent of determining if commercially reasonable thermal treatments would have sufficient lethality for *Salmonella sp.* It is expected that increasing the number of samples and extending the temperature range would result in some changes in the slope of the line, especially at lower temperatures (Cotterill et al., 1973). Based on concerns for the interior quality and their use in cooking, the practical upper temperature range would probably be less than 60°C. At temperatures in the range of 55 to 65°C, Cotterill et al. (1973) generally found linear TDTC for destruction of *S. oranienburg*. It is anticipated that the F_0 line for other forms of *Salmonella* in shell egg are also linear over that temperature range.

It is established that different strains of *Salmonella*, the type of egg product, and other environmental conditions will effect the thermal inactivation of *Salmonella*. Shah et al. (1991) presented D values for 17 strains of *S. enteritidis* in whole egg ranging from 13.7 to 31.3 seconds at 60°C. The average D was 19.2±5.4 sec. and was reported to be similar to previous data. Cotterill et al. (1973) and USDA (1969) provide data showing the influence of egg product type, pH, salt, and sugar on the thermal resistance of *Salmonella sp.* When evaluating the thermal resistance of *Salmonella* in intact shell eggs, the location of the bacteria within the egg becomes important. The thermal resistance of *Salmonella* in different egg products is as follows: plain yolk>whole egg or pH 7 egg white>pH 9 egg white (USDA, 1969). Therefore, increased thermal treatments would be required for plain yolk over whole egg or pH 7 egg white or pH 9 egg white.

In this study, the culture was placed in the egg white near the surface of the yolk. The consensus of those actively studying *S. enteritidis* infection of shell eggs is that the bacteria is found in the egg white of naturally infected eggs produced by infected hens (Gast and Beard, *J. Food Prot.,* **55**:152-156 (1991); Beard, *Egg Industry,* **92**:3337 (1992)). The "Apparent F_0" line was plotted in Figure 1, a redrawing of Figure 6 from the Egg Pasteurization Manual (USDA, 1969). This allows a visual evaluation of the thermal processes applied to intact shell eggs relative to accepted minimal pasteurization processes for liquid egg products.

When comparing the "Apparent F_0" line and actual processes to the lines for pH 9 egg white and whole egg or pH 7 egg white, the shell egg processes seem to be more than adequate to achieve reductions of *S. enteritidis* sufficient for an accepted pasteurization process for protection of public health. The pH of the egg whites in this study ranged from 8.4 to 8.6 which is typical for shell eggs the age of those used in this study.

Although natural infections of the yolk are not expected at the time of ovulation, it is clear that under adverse handling conditions, *S. enteritidis* can be introduced into the egg and grow to very high numbers in the yolk (Hammack *et al.*, *Poultry Science,* **72**:373-377 (1993)). At 56°C (134°F), if the cells were in the yolk, the minimum holding time would be 36.42 minutes for an adequate pasteurization process. Since the "Apparent F_0" line crosses the USDA yolk pasteurization line at about 134°F, it is therefore preferred that thermal treatments for shell eggs at temperatures above 134°F be selected.

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In addition to the F_0 analysis described above, an equivalent point analysis of the time-temperature curve of the thermal treatment imparted to the shell egg may be utilized to determine the total thermal treatment imparted various locations in the shell egg. A temperature probe was inserted into shell eggs in the aqueous solution at various depths into the egg. Temperatures were taken in the albumen at the yolk/albumen interface and in the yolk. These temperatures were taken using a hypodermic needle probe model HYP4-16-1-1/2-100-EU-48-RP manufactured by BIOMEGA® of Stamford Connecticut. The probe was inserted into the egg through a cork which was glued to the egg and prevented water from entering the egg through the aperture created by the probe. A DAYTRONIC® System 10 data acquisition unit was connected through an RS-232 serial connection to a personal computer. Temperature measurements were taken every 5 seconds and recorded. A representative thermal curve for a thermal treatment to the shell eggs is shown in Figure 2. To evaluate the equivalent point for the thermal curve shown in Figure 2, the thermal reduction relationship (G_{Ea}) is calculated using the following equation:

T 10170

$$G_{Ea} = \int_0^{t_{final}} e^{-\frac{Ea}{RT(t)}} dt$$

where Ea is the activation energy (J/mol), R is the Universal Gas Constant (8.314 J/mol,K), T(t) is temperature as a function of time (°K) and t_{final} is the final processing time (s). This integration process is then repeated for a number of activation energies (Ea). Each G_{Ea} value defines a line of equivalent thermal treatments for that particular activation energy (Ea). The intersection of the lines defined by the G_{Ea}'s is the equivalent point of the thermal process. (Swartzel, 1986, *J. Agric. Food Chem.*, **34**:397).--

B10

Please replace the paragraph beginning at page 17, line 4, with the following rewritten paragraph:

--Use of the time and temperature relationships discussed above should result in a shell egg which may be guaranteed to be *Salmonella* negative. As used herein *Salmonella* negative means a negative result indicating the absence of harmful *Salmonella* as determined by USDA approved methods of *Salmonella* testing. This insured *Salmonella* negative shell egg is referred to herein as a pasteurized shell egg.--

B11

Please replace the paragraph beginning at page 17, line 14, with the following rewritten paragraph:

--Quality and functional attributes of shell eggs heated at 56.75 and 57.5°C with and without oiling are summarized in Table 2. The expected ability of oiling egg shells to maintain fresh egg pH and interior quality is evident. The egg white pH of the oiled eggs is clearly lower than for the unoiled eggs regardless of storage temperature. The thermal treatments did

B12

not seem to have an effect on egg white pH, but did seem to have an impact on interior quality as indicated by the Haugh unit values. For the non-thermally treated eggs, oiling held egg white pH and resulted in higher Haugh values at both storage temperatures. Oiling the thermally treated eggs appeared to help maintain interior quality if they were stored at room temperature (22.2°C). The thermal treatments alone, provided good protection of interior quality. All thermally treated eggs regardless of oiling or storage temperature would be considered high A or AA quality grades. There seemed to be less correlation of egg white pH with interior quality than might have been expected. This is particularly so when comparing the egg white pH and Haugh units of oiled and unoiled eggs. That result suggests the thermal treatments are stabilizing interior quality independently of deterioration mechanisms related to change in egg white pH. Funk U.S. Pat. No. 2,423,233 (1947) claimed that heating shell eggs for 5 to 40 minutes at temperatures of 60 to 43.4°C, respectively, would maintain interior quality without impairing the whipping qualities. However, he did not define quality or whipping qualities.--

Please replace the table beginning at page 18, line 9, with the following rewritten table:

--Table 3: Quality and Functional attributes of thermally treated shell eggs with and without oiling four weeks storage at 22.2 or 7.2°C.

	Egg White pH		Haugh Unit		Whip Volume[a]		Whip Time[b]	
No Oil	22.2C	7.2C	22.2C	7.2C	22.2C	7.2C	22.2C	7.2C
No Heat	9.3	9.2	20	60	1,000	900	40	45
56.75C, 36 min.	9.2	8.9	78	82	550	650	220	110
57.5C, 23 min.	9.2	9.1	74	82	750	600	280	130
Oiled								
No Heat	8.0	8.1	58	70	950	800	45	45
56.75C, 36 min.	7.9	8.2	80	80	550	650	190	200
57.5C, 23 min.	8.0	8.1	81	82	600	700	200	210

[a]Whip Volume in ml.
[b]Whip Time in sec.
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